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Description

The present invention relates to a pharmaceutical agent for the treatment of myelogenous leukemia that contains a human granulocyte colony stimulating factor (hereinafter abbreviated as G-CSF) as the active ingredient.

In spite of the development of drugs as typified by chemotherapeutics or the advances in therapeutic regimens such as bone marrow transplantation, leukemia still remains a disease that is difficult to completely cure as is manifested by its mortality rate that has not decreased for the past 20 years [see Igaku no Ayumi, 128, 13, 1984 (hereinafter abbreviated as Ayumi), 867 - 873]. On the other hand, efforts to develop anti-leukemic drugs are being made at a remarkable pace and the rate of complete remission of adult acute myelogenous leukemia (AML) in patients who have received a treatment by multiple chemotherapeutics (complete remission of this disease is indicated by normalization in the quality and quantity of blood cells in peripheral blood and bone marrow and by the disappearance of the subjective symptoms and objective physical findings due to leukemia) has reportedly reached levels of 80% or more (see Ayumi, 994 - 998). Unfortunately, however, the efforts so far made to achieve complete healing of AML have proved very unrewarding as evidenced by its high mortality rate and there is no efficacious drug available for the treatment of chronic myelogenous leukemia (CML) (see Ayumi, 1005 - 1011).

Under these circumstances, studies are being undertaken to develop a new type of leukemia-treating drug that utilises biological response moderators (BRM) such as immunotherapeutics (Ayumi, 1050 - 1055), differentiation inducers (Ayumi, 1059 - 1063) and interferons (Ayumi, 1056 - 1058). However, no drug has been proposed to date that is capable of complete healing of leukemia.

An interesting finding has recently been reported; a substance capable of inducing the differentiation of mouse myelogenous leukemia cell, WEHI-3B, was purified from the supernatant of the culture of the lung tissue of mice and it was found to be identical with a mouse G-CSF (Nicola, N.A. et al.; J. Biol. Chem., 258, 9017 - 9023, 1983). This G-CSF is a BRM that acts on granulocyte precursor cells to promote their differentiation and growth to granulocytes (see, for example, Metcalf et al.; Exp. Hematol., 1, 185, 1973).

The present inventors have conducted intensive studies on human G-CSF and filed patent applications on their success in achieving large-scale preparation of a pure human G-CSF (see EP No. 85 10 9336.9 (EP-A 169566), EP No. 86 11 3671.1 (EP-A 217404) and EP No. 86 90 1138.7 (EP-A 215126, WO 8604605). One of the CSFs prepared by the present inventors is a human G-CSF derived from CHU-2. When this CSF was administered to mice, the number of mature neutrophils in peripheral blood showed a marked increase (see Experiment 1 described later in this specification). An experiment was also conducted to investigate the efficacy of G-CSF for treating radiation-induced leukemia in SJL/J mouse models. A significant life-span prolonging effect was achieved by G-CSF (see Experiment 2). The SJL/J mice are models in which leukemia was induced by irradiation and they enable more reliable simulation of leukemia than conventional models in which leukemia cell lines are transplanted intraperitoneally. The SJL/J mice are highly prone to develop myelogenous leukemia (15 - 20% incidence upon exposure to irradiation of total dose of 300 R). In Experiment 2, it was also demonstrated that administration of G-CSF caused an increase in the proportion of mature neutrophils and this was confirmed by classification of peripheral neutrophilic cells on the basis of their maturity. These mature neutrophils may have originated from (1) differentiated leukemic cells or from (2) the differentiation and growth of normal cells that remained in leukemic mice. Whichever the case may be, the life-span prolonging effect of G-CSF is most probably due to its ability to increase the number of peripheral mature neutrophils in leukemic mice. Since the results of Experiment 2 were obtained from models that closely simulate the development of leukemia, it may be safely concluded that they demonstrate the usefulness of G-CSF as a leukemia treating agent.

The present invention has been accomplished on the basis of the aforementioned findings.

The present invention provides the use of a human G-CSF for the preparation of a pharmaceutical agent for the treatment of myelogenous leukemia.

The human G-CSF used as the active ingredient of the pharmaceutical agent of the present invention may be derived from any origin that is capable of producing an isolated human G-CSF of high purity. It is preferable to use the following two types of human G-CSF that were obtained by the methods on which patent applications were previously applied by the present inventors:

(i) human G-CSF having the following physicochemical properties:

- i) molecular weight: $19,000 \pm 1,000$ as measured by electrophoresis through a sodium dodecylsulphate - polyacrylamide gel;
- ii) isoelectric point: having at least one of the three isoelectric points, $pI = 5.5 \pm 0.1$, $pI = 5.8 \pm 0.1$, and $pI = 6.1 \pm 0.1$;
- iii) ultraviolet absorption: having a maximum absorption at 280 nm and a minimum absorption at 250

nm;

iv) amino acid sequence of the 21 residues from N terminus:

5 H₂N - Thr - Pro - Leu - Gly - Pro - Ala - Ser - Ser -
 Leu - Pro - Gln - Ser - Phe - Leu - Leu - Lys - Cys -
 Leu - Glu - Gln - Val -

10 (2) a human G-CSF having a polypeptide represented by all or part of the following amino acid sequence:

15 (Met)_n Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro
 Gln Ser Phe Leu Leu Lys Cys Leu Glu Gln Val
 Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln

20 Glu Lys X Cys Ala Thr Tyr Lys Leu Cys His
 Pro Glu Glu Leu Val Leu Leu Gly His Ser Leu
 Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro
 25 Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser
 Gln Leu His Ser Gly Leu Phe Leu Tyr Gln Gly
 Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro Glu
 30 Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp
 Val Ala Asp Phe Ala Thr Thr Ile Trp Gln Gln
 Met Glu Glu Leu Gly Met Ala Pro Ala Leu Gln
 35 Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser
 Ala Phe Gln Arg Arg Ala Gly Gly Val Leu Val
 Ala Ser His Leu Gln Ser Phe Leu Glu Val Ser
 40 Tyr Arg Val Leu Arg His Leu Ala Gln Pro

(where X is Leu or Leu-Val-Ser-Glu; and n is 0 or 1).

Most preferably, either of the two types of human G-CSF takes on the form of glycoprotein having a sugar chain portion.

45 The G-CSF of type (1) may be prepared by either of the methods described in EP No. 85 10 9336.9 (EP-A 169566) and EP No. 86 11 3671.1 (EP-A 217404). The former application describes a method of isolating the desired human G-CSF from the supernatant of the culture of a cell strain, CHU-1, that was derived from human oral cavity cancer and which has been deposited with Collection Nationale de Cultures de Microorganismes, Institut Pasteur, France under C.N.C.M. Accession Number I-315. The latter application describes a method of isolating the desired human G-CSF from the supernatant of the culture of a cell strain, CHU-2, that was also derived from human oral cavity cancer and which has been deposited with C.N.C.M. under Accession Number I-483. For further details of the two methods, see the specifications of the respective applications.

55 The G-CSF of type (2) may be prepared by either of the methods described in EP No. 86 90 1138.7 (EP-A 215126). All of these methods rely on "DNA recombinant technology". The methods described in the first two applications use *E. coli* and other procaryotic cells as host cells, and those given in the other two applications employ animal cells as host cells. For further details of these methods, see the specifications of the respective applications.

The most desirable type of G-CSF which assumes the form of a glycoprotein having a sugar chain portion can be produced by the method using animal cells as hosts.

The human G-CSF obtained by either of the methods outlined above may be stored in a frozen state or after being dehydrated by such means as freeze-drying or vacuum drying. If desired, the human G-CSF may be dissolved in an appropriate buffer, followed by aseptic filtration through a Millipore filter or any other suitable means to formulate an injection.

The pharmaceutical agent for the treatment of myelogenous leukemia of the present invention may contain the pharmaceutical carrier or excipient necessary to assist in its formulation in a dosage form suitable for administration to humans. If desired, a stabilizer and an anti-adsorption agent may also be incorporated in this agent.

The level of dosage and the frequency of administration of the human G-CSF in the pharmaceutical agent of the present invention may be determined in consideration of the severity of the disease to be treated; typically, a dosage containing 0.1 - 500 μ g, preferably 5 - 100 μ g, of human G-CSF may be administered to an adult at a frequency of one to seven times a week.

The pharmaceutical agent of the present invention which is intended to be used as a curative for myelogenous leukemia is effective not only for increasing the number of peripheral mature neutrophils in patients with myelogenous leukemia but also for prolonging their lives. Therefore, the present invention will hold increased promise for the treatment of myelogenous leukemia which has been impossible to cure completely by conventional therapeutic drugs or regimens.

Examples

The following reference example, experimental examples and working examples are provided for the purpose of illustrating the preparation of G-CSF, its pharmacological effects and its formulation in various dosage forms, respectively, but it should be understood that the scope of the present invention is by no means limited by these examples.

Reference Example: The Preparation of human G-CSF using animal cells (mouse C127 cells)

Plasmid PTN-V2 was obtained by the procedures described in Examples 1 - 12 of Japanese Patent Application No. 269456/1985 (EP-A 215126), and subsequently treated with BamHI as follows. Twenty micrograms of the plasmid pTN-V2 was dissolved in 100 μ l of a reaction solution [10 mM Tris-HCl (pH 8.0), 7 mM MgCl₂, 100 mM NaCl, 2 mM 2-mercaptoethanol and 0.01% BSA] and treated with 20 units of BamHI (Takara Shuzo Co., Ltd.), followed by treatments with phenol and ether, and precipitation with ethanol.

Mouse C127 cells were grown in a Dulbecco's minimal essential medium containing 10% bovine foetal serum (Gibco). The C127 cells growing on plates (5 cm²) were transformed with 10 μ g per plate of the separately prepared DNA by the calcium phosphate procedure [see Haynes, J. & Weissmann, C., Nucleic Acids Res., 11, 687 - 706 (1983)]. After treatment with glycerol, the cells were incubated at 37°C for 12 hours.

The incubated cells were transferred onto three fresh plates (5 cm²) and the media changed twice a week. At day 16, the foci were transferred onto fresh plates and subjected to serial cultivation on a Dulbecco's minimal essential medium containing 10% bovine foetal serum (Gibco), so as to select clones having high G-CSF production rate. These clones produced G-CSF at a level of approximately 1 mg/l.

For the methods of recovering, purifying and assaying the so obtained G-CSF, see the pertinent Examples shown in the specification of Japanese Patent Application No. 269456/1985 (EP-A 215126).

Experiment I: Increase in the number of peripheral mature neutrophils upon G-CSF administration

C57BL mice (male, 8-week-old) were divided into two groups. 0.1 ml of a control sample (a physiological saline solution containing 1% n-propanol and 10% serum from C57BL mice) was administered subcutaneously once a day to one group (control group). To the other group (CSF-treated group), 0.1 ml of a CSF sample (a physiological saline solution containing 2.5 μ g of CHU-2 derived G-CSF, 1% n-propanol and 10% serum from C57BL mice) was administered subcutaneously once a day. On predetermined days, 4 mice were sampled randomly from each group and the number of leukocytes in blood samples taken from the orbital vein was determined with a micro cell counter (Model CCI80 of Toa Medical Electronics Co., Ltd.) In a separate step, blood smears were prepared and Giemsa staining was conducted in order to determine the proportion of peripheral neutrophils in 200 leukocytes under the microscope. The number of peripheral neutrophils in each group was calculated by the following formula:

Peripheral neutrophiles = (peripheral leukocyte count) \times (proportion of neutrophils in leukocytes)

In this experiment, no mouse was bled on more than one occasion. A group of C57BL mice to which no injection was given at all were treated by the same procedures to obtain the values for day 0. The results are shown in Table 1.

Table 1

Days	(number of measurements, n = 4)	
	Peripheral neutrophile count (cells/mm ³)	
	Control group	CSF treated group
0	1213 \pm 354	1213 \pm 354
2	769 \pm 219	3534 \pm 596
5	989 \pm 158	3867 \pm 482**
8	889 \pm 44	2724 \pm 557**
11	696 \pm 81	3600 \pm 407***
15	639 \pm 95	5020 \pm 326***
P: ***<0.001<**<0.01<*<0.05		

As Table 1 shows, the human G-CSF derived from CHU-2 has the ability to increase the number of mature neutrophils in peripheral blood.

Experiment 2: Anti-leukemic effect of G-CSF in SJL/J leukemic mice.

SJL/J mice (male, 7-week-old) were exposed to irradiation of total dose of 300 R. For a subsequent period of 120 days, blood samples were taken as in Experiment 1 once every 30 days, and once every 10 days in the following period. The numbers of peripheral erythrocytes and leukocytes in each blood sample were counted, and at the same time blood smears were also prepared. Leukemia was considered to have developed if at least two of the following three criteria were satisfied: 1) the peripheral leukocyte count was 30,000 cells/mm³ or more; 2) the peripheral erythrocyte count was 5×10^6 cells/mm³ or less; and 3) the appearance of leukoblasts in peripheral blood was apparent.

To each of the mice that had developed leukemia, 0.1 ml of the control or G-CSF sample shown in Experiment 1 was administered daily through a subcutaneous route until the mice died. The period for which the mice remained alive following injection of either sample was compared. Another group of leukemic mice were treated in the same manner and blood samples were taken from the orbital vein on predetermined days so as to determine the peripheral leukocyte count and the maturity of neutrophilic granulocytes. All leukemic mice subjected to these tests were found to have experienced swelling of spleen and infiltration of leukemic cells into spleen, which served as another evidence that these mice were truly leukemic. The test results are shown below in (1) and (2) (Table 2).

(1) Days of survival

Control group: 9 ± 1.47

CSF treated group: 28.75 ± 1.93

(number of measurements, n = 4; P < 0.001)

Table 2

	CSF treated mice								Control mice			
	-15	0	4	9	12	18	27	32	-14	0	4	8
Days after development of leukemia												
Polymorphonuclear leukocytes*	84	10	9	19	45	43	18	12	88	12	11	10
Band cell metamyelocytes*	16	64	50	29	22	38	42	51	12	53	47	52
Myelocytes*	0	18	21	31	23	11	23	20	0	23	25	13
Promyelocytes*	0	8	20	21	10	8	13	11	0	12	17	8
Myeloblasts*	0	0	0	0	0	0	4	6	0	0	0	17

* Numbers were determined for 100 neutrophilic cells.

As the data in (1) show, administration of G-CSF was effective in prolonging the life-span of leukemic mice by a significant degree. As is clear from the data in (2) (Table 2) which show the numbers of cells at different stages of maturity of neutrophilic cells, the CSF-treated mice had more polymorphonuclear leukocytes (mature neutrophils) than the control mice. It is believed that this ability of CSF to increase the number of peripheral mature neutrophils is responsible for the life-span prolonging effect of G-CSF. It is

worth particular attention that the results of Experiment 2 were obtained with an animal model that would closely simulate the development of leukemia in the actual case.

Example 1

The human G-CSF prepared in the Reference Example was rendered germ-free and frozen at -20°C . The frozen fraction was worked up to prepare an injection.

Example 2

The human G-CSF prepared in the Reference Example was aseptically charged in 5 ml portions in 10 ml vials and freeze-dried at -20°C , with the vials being subsequently closed with rubber stopper. The so obtained freeze-dried products were worked up to prepare an injection.

Claims

1. The use of human granulocyte colony stimulating factor for preparing a pharmaceutical composition for treating myelogenous leukemia, said human granulocyte colony stimulating factor having the following physicochemical properties:

- i) molecular weight: $19,000 \pm 1,000$ as measured by electrophoresis through a sodium dodecylsulphate - polyacrylamide gel;
- ii) isoelectric point: having at least one of the three isoelectric points, $pI = 5.5 \pm 0.1$, $pI = 5.8 \pm 0.1$, and $pI = 6.1 \pm 0.1$;
- iii) ultraviolet absorption: having a maximum absorption at 280 nm and a minimum absorption at 250 nm;
- iv) amino acid sequence of the 21 residues from N terminus:

$\text{H}_2\text{N} - \text{Thr} - \text{Pro} - \text{Leu} - \text{Gly} - \text{Pro} - \text{Ala} - \text{Ser} - \text{Ser} -$
 $\text{Leu} - \text{Pro} - \text{Gln} - \text{Ser} - \text{Phe} - \text{Leu} - \text{Leu} - \text{Lys} - \text{Cys} -$
 $\text{Leu} - \text{Glu} - \text{Gln} - \text{Val} -$

2. The use of human granulocyte colony stimulating factor for preparing a pharmaceutical composition for treating myelogenous leukemia, said human granulocyte colony stimulating factor representing all or part of the amino acid sequence shown below:

	(Met) _n	Thr	Pro	Leu	Gly	Pro	Ala	Ser	Ser	Leu	Pro
	Gln	Ser	Phe	Leu	Leu	Lys	Cys	Leu	Glu	Gln	Val
5	Arg	Lys	Ile	Gln	Gly	Asp	Gly	Ala	Ala	Leu	Gln
	Glu	Lys	X	Cys	Ala	Thr	Tyr	Lys	Leu	Cys	His
	Pro	Glu	Glu	Leu	Val	Leu	Leu	Gly	His	Ser	Leu
10	Gly	Ile	Pro	Trp	Ala	Pro	Leu	Ser	Ser	Cys	Pro
	Ser	Gln	Ala	Leu	Gln	Leu	Ala	Gly	Cys	Leu	Ser
	Gln	Leu	His	Ser	Gly	Leu	Phe	Leu	Tyr	Gln	Gly
15	Leu	Leu	Gln	Ala	Leu	Glu	Gly	Ile	Ser	Pro	Glu
	Leu	Gly	Pro	Thr	Leu	Asp	Thr	Leu	Gln	Leu	Asp
	Val	Ala	Asp	Phe	Ala	Thr	Thr	Ile	Trp	Gln	Gln
	Met	Glu	Glu	Leu	Gly	Met	Ala	Pro	Ala	Leu	Gln
20	Pro	Thr	Gln	Gly	Ala	Met	Pro	Ala	Phe	Ala	Ser
	Ala	Phe	Gln	Arg	Arg	Ala	Gly	Gly	Val	Leu	Val
	Ala	Ser	His	Leu	Gln	Ser	Phe	Leu	Glu	Val	Ser
25	Tyr	Arg	Val	Leu	Arg	His	Leu	Ala	Gln	Pro	

(where X is Leu or Leu-Val-Ser-Glu; and n is 0 or 1).

30 Revendications

1. Utilisation du facteur de stimulation des colonies de granulocytes humains pour préparer une composition pharmaceutique destinée à traiter la leucémie myélogène, ledit facteur de stimulation des colonies de granulocytes humains ayant les propriétés physico-chimiques suivantes :

- i) masse moléculaire : 19000 ± 1000 , telle que mesurée par électrophorèse sur gel de dodécylsulfate de sodium polyacrylamide;
- ii) point isoélectrique : ayant au moins un des trois points isoélectriques $pI = 5,5 \pm 0,1$, $pI = 5,8 \pm 0,1$ et $pI = 6,1 \pm 0,1$;
- iii) absorption dans l'ultraviolet : ayant une absorption maximum à 280 nm et une absorption minimum à 250 nm;
- iv) séquence d'acides-amino des 21 résidus, à partir de la terminaison N :

H_2N - Thr - Pro - Leu - Gly - Pro - Ala - Ser - Ser -
 Leu - Pro - Gln - Ser - Phe - Leu - Leu - Lys - Cys -
 Leu - Glu - Gln - Val -

2. Utilisation du facteur de stimulation des colonies de granulocytes humains pour préparer une composition pharmaceutique destinée à traiter la leucémie myélogène, ledit facteur de stimulation des colonies de granulocytes humains étant représenté par tout ou partie de la séquence d'acides-amino précisée ci-dessous :

	(Met) _n	Thr	Pro	Leu	Gly	Pro	Ala	Ser	Ser	Leu	Pro
	Gln	Ser	Phe	Leu	Leu	Lys	Cys	Leu	Glu	Gln	Val
5	Arg	Lys	Ile	Gln	Gly	Asp	Gly	Ala	Ala	Leu	Gln
	Glu	Lys	X	Cys	Ala	Thr	Tyr	Lys	Leu	Cys	His
	Pro	Glu	Glu	Leu	Val	Leu	Leu	Gly	His	Ser	Leu
	Gly	Ile	Pro	Trp	Ala	Pro	Leu	Ser	Ser	Cys	Pro
10	Ser	Gln	Ala	Leu	Gln	Leu	Ala	Gly	Cys	Leu	Ser
	Gln	Leu	His	Ser	Gly	Leu	Phe	Leu	Tyr	Gln	Gly
	Leu	Leu	Gln	Ala	Leu	Glu	Gly	Ile	Ser	Pro	Glu
15	Leu	Gly	Pro	Thr	Leu	Asp	Thr	Leu	Gln	Leu	Asp
	Val	Ala	Asp	Phe	Ala	Thr	Thr	Ile	Trp	Gln	Gln
	Met	Glu	Glu	Leu	Gly	Met	Ala	Pro	Ala	Leu	Gln
20	Pro	Thr	Gln	Gly	Ala	Met	Pro	Ala	Phe	Ala	Ser
	Ala	Phe	Gln	Arg	Arg	Ala	Gly	Gly	Val	Leu	Val
	Ala	Ser	His	Leu	Gln	Ser	Phe	Leu	Glu	Val	Ser
25	Tyr	Arg	Val	Leu	Arg	His	Leu	Ala	Gln	Pro	

(ou x est Leu ou Leu-Val-Ser-Glu et n est égal à 0 ou à 1).

30 Patentansprüche

1. Verwendung eines menschlichen Granulozyten-Kolonie-stimulierenden Faktors zur Herstellung eines Arzneimittels für die Behandlung von myelogener Leukämie, wobei der menschliche Granulozyten-Kolonie-stimulierende Faktor die folgenden physikochemischen Eigenschaften aufweist:

- i) Molekulargewicht:
19 000 ± 1000, gemessen durch Elektrophorese in einem Natriumdodecylsulfat-Polyacrylamidgel;
- ii) Isoelektrischer Punkt:
mindestens einen der drei isoelektrischen Punkte, pI = 5,5 ± 0,1, pI = 5,8 ± 0,1 und pI = 6,1 ± 0,1;
- iii) Ultraviolettabsorption:
Ein Absorptionsmaximum bei 280 nm und ein Absorptionsminimum bei 250 nm;
- iv) Aminosäuresequenz der 21 Reste vom N-terminalen Ende:

H_2N - Thr - Pro - Leu - Gly - Pro - Ala - Ser - Ser -
 Leu - Pro - Gln - Ser - Phe - Leu - Leu - Lys - Cys -
 Leu - Glu - Gln - Val -

2. Verwendung eines menschlichen Granulozyten-Kolonie-stimulierenden Faktors zur Herstellung eines Arzneimittels für die Behandlung von myelogener Leukämie, wobei der menschliche Granulozyten-Kolonie-stimulierende Faktor die gesamte oder einen Teil der nachstehend gezeigten Aminosäuresequenz aufweist:

	(Met)	Thr	Pro	Leu	Gly	Pro	Ala	Ser	Ser	Leu	Pro
	Gln	Ser	Phe	Leu	Leu	Lys	Cys	Leu	Glu	Gln	Val
5	Arg	Lys	Ile	Gln	Gly	Asp	Gly	Ala	Ala	Leu	Gln
	Glu	Lys	X	Cys	Ala	Thr	Tyr	Lys	Leu	Cys	His
	Pro	Glu	Glu	Leu	Val	Leu	Leu	Gly	His	Ser	Leu
	Gly	Ile	Pro	Trp	Ala	Pro	Leu	Ser	Ser	Cys	Pro
10	Ser	Gln	Ala	Leu	Gln	Leu	Ala	Gly	Cys	Leu	Ser
	Gln	Leu	His	Ser	Gly	Leu	Phe	Leu	Tyr	Gln	Gly
	Leu	Leu	Gln	Ala	Leu	Glu	Gly	Ile	Ser	Pro	Glu
15	Leu	Gly	Pro	Thr	Leu	Asp	Thr	Leu	Gln	Leu	Asp
	Val	Ala	Asp	Phe	Ala	Thr	Thr	Ile	Trp	Gln	Gln
	Met	Glu	Glu	Leu	Gly	Met	Ala	Pro	Ala	Leu	Gln
20	Pro	Thr	Gln	Gly	Ala	Met	Pro	Ala	Phe	Ala	Ser
	Ala	Phe	Gln	Arg	Arg	Ala	Gly	Gly	Val	Leu	Val
	Ala	Ser	His	Leu	Gln	Ser	Phe	Leu	Glu	Val	Ser
25	Tyr	Arg	Val	Leu	Arg	His	Leu	Ala	Gln	Pro	

(wobei X Leu oder Leu-Val-Ser-Glu ist und n den Wert 0 oder 1 hat).

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